Mathematical Models of Biochemical Oscillations

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Abstract

The goal of this paper is to explain the mathematics involved in modeling biochemical oscillations. We first discuss several important biochemical concepts fundamental to the construction of descriptive mathematical models. We review the basic theory of differential equations and stability analysis as it relates to two-variable models exhibiting oscillatory behavior. The importance of the Hopf Bifurcation will be discussed in detail for the central role it plays in limit cycle behavior and instability.

Once we have exposed the necessary mathematical framework, we consider several specific models of biochemical oscillators in three or more variables. This will include a detailed analysis of Goodwin's equations and their modification first studied by Painter. Additionally, we consider the consequences of introducing both distributed and discrete time delay into Goodwin's model. We will show that the presence of distributed time lag modifies Goodwin's model in no significant way. The final section of the paper will discuss discrete time lag in the context of a minimal model of the circadian rhythm.

In the main, this paper will address mathematical, as opposed to biochemical, issues. Nevertheless, the significance of the mathematics to the biochemistry will be considered throughout.

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1. Biochemical Oscillations

Albert Goldbeter begins his text on biochemical oscillations [1] by describing rhythmic phenomena as "among the most conspicuous properties of living systems... occurring at all levels of biological organization, from unicellular to multicellular organisms, with periods ranging from fractions of seconds to years." He continues to relate that the mechanisms involved "often remain unclear" but that significant progress has been made in the past 100 years. Constructing viable models of complex rhythmic phenomena can often be quite a challenge.

Mathematical models of biochemical systems which exhibit oscillation have traditionally relied on underlying *feedback loops*. *Feedback* is a propagation of effects among the components of a biochemical system in which one chemical component inevitably predicts an increase or decrease in its own rate of production. A feedback loop can be represented diagramatically as in Figure 1. Each of the arrows directed

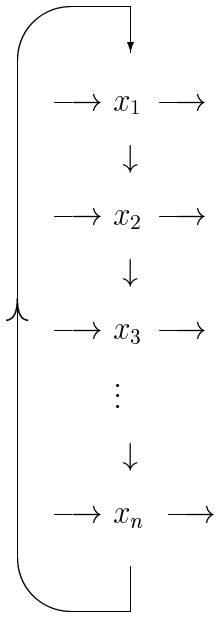

Figure 1. A Feedback Loop: Each arrow directed into x_i (and out of x_i) represents processes contributing to the synthesis (and degradation) of x_i, while each of the arrows directed from x_i to x_{i+1} represents either a negative or positive effect to the next variable in the chain.

into x_i from the left and *out* of x_i from the right represents all processes contributing

to the synthesis and degradation of x_i, respectively. Each of the arrows directed from x_i to x_{i+1} represents a *negative* or *positive* effect to the rate of production of the variable x_{i+1}. A negative (positive) effect can be either an inhibition (activation) of the processes contributing to the synthesis or an activation (inhibition) of the processes contributing to the degradation of x_{i+1}. Mathematically, a negative effect from x_i to x_{i+1} can be represented by the expression $\frac{\partial}{\partial x_i}(\frac{dx_{i+1}}{dt}) < 0$ while a positive effect can be represented as $\frac{\partial}{\partial x_i}(\frac{dx_{i+1}}{dt}) > 0$. The *identity* of a feedback loop as *positive* or *negative* depends on the sign of the product[1]

$$\prod_{i=1}^{n} \frac{\partial}{\partial x_i}(\frac{dx_{i+1}}{dt}) \tag{1.1}$$

The feedback loop is negative or positive depending on whether this product is negative or positive. While negative feedback generally leads to *homeostasis* or stable steady state solutions, positive feedback tends to destabilize a system.

One of the first and most prominent examples of negative feedback in a biochemical oscillator was described by Brian C. Goodwin and first given analytic treatment by Griffith [3] in 1968. Goodwin's model comprises a set of differential equations which are linear save a term controlling the synthesis of the first chemical component in the feedback loop. This nonlinear expression is capable of causing spontaneous oscillation. Unfortunately, the conditions which force the system into oscillation are biochemically unreasonable. In 1984, Bliss and Painter [4] proposed a model based on Goodwin's equations which undergoes a transition to oscillation under much more reasonable conditions. The presence of an additional nonlinear term in the rate law of the last component of the chain is sufficient to cause oscillation. This nonlinear term is, in fact, a Michaelis-Menten expression which represents a more biochemically reasonable assumption about the binding of the chemicals involved.

In 1977, MacDonald [5] analyzed a generalization of Goodwin's equations which incorporates *time delay.* The dependency of one chemical component on the history of another chemical component can also force the system into oscillation. When this dependency is distributed, the generalized model is, in fact, equivalent to Goodwin's equations with an increased number of variables.

[1]The last term of the product (with subscript $n+1$) refers to the original component in the loop. That is, the subscripts are taken modulo n.

There is considerable motivation for modeling such biochemical behavior. This motivation is nowhere more evident than in the study of *circadian rhythms*, endogenous biological oscillations with periods of about 24 hours, where recent advances in molecular biology have "uncovered stunning similarities among the molecular circuitries of circadian clocks" in several organisms [6]. As new details in the molecular biology arise, we may build more and more accurate models to explain and predict biochemical behavior.

2. Basic Concepts

A few ideas from biochemistry are requisite to any discussion of biochemical oscillations. [2] We define a *biochemical reaction* to be an enzyme-catalyzed chemical reaction. A catalyst is a substance which causes an increase in the rate of a chemical reaction. In living cells, catalysts are most commonly proteins called *enzymes.* Molecules of RNA known as *ribozymes* can also act as catalysts, but they play no role in our discussion. A biochemical system is therefore characterized as a network of chemical reactions whose dynamic behavior is determined by the functionality of the protein catalysts involved.

The *dynamics* of a biochemical system are characterized by *rate laws* which describe the rate at which the concentrations of the chemicals in the system change. This rate will depend on the concentrations of other chemical components present in the system. The characterization of precisely how rate laws depend on chemical concentrations is called *chemical kinetics.* Rate laws can be of several different *orders*, depending on the number of chemicals involved in the reaction.

Consider a biochemical system involving a single chemical X whose concentration is denoted x. The change in x is given by $\dfrac{dx}{dt}$ which, in this case, depends only on an expression involving x. Trivially, this could be constant, i.e. $\dfrac{dx}{dt} = \pm k$, in which case the concentration is a linear function of time. More interestingly, consider the *chemical mechanism*

$$A + X \xrightarrow{k_a} 2X$$

[2] The explanations found in this section come from a basic text on biochemistry [2].

This says that X combines with another substance in a reaction which produces two molecules of X. Thus, X tends to catalyze its own production by increasing the amount of X available for reaction. This type of behavior is called *autocatalysis*. The rate law for X takes the form $\frac{dx}{dt} = k_a ax$. Note that $\frac{\partial}{\partial x}(\frac{dx}{dt}) = k_a a > 0$ for positive values of the concentration a. In fact, the general mathematical characterization of an autocatalytic chemical X is $\frac{\partial}{\partial x}(\frac{dx}{dt}) > 0$.

In contrast, a single chemical X will tend to *degrade* at a rate proportional to the concentration x in the absence of reaction with other chemicals. This degradation can be represented mathematically by $\frac{\partial}{\partial x}(\frac{dx}{dt}) < 0$.

The proportionality evident in the rate laws discussed above is general and is a result of the *Law of Mass Action* which states that the rate of change in the concentration of a chemical component of a biochemical system is proportional to some product of concentrations of the chemicals present in the system. When the rate law takes the form $-kx$, as in a simple chemical degradation, we say the reaction is described by *first order kinetics* because only one concentration contributes to the product.

On the other hand, consider the enzymatic conversion of substance X into a product Y described by

$$X + Z \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \overset{k_3}{\rightarrow} Y + Z \tag{2.1}$$

In the process of converting X to Y, the enzyme Z combines with X to form the *substrate-enzyme complex* C. While in this complex, X is converted to Y by the action of the enzyme. Upon separation, a new substance Y appears along with the original enzyme, which is cycled back so that it may again react with X. For each chemical present in the system, a rate law can be formed based on the Law of Mass Action to describe the change in concentration. Let us consider the rate law for X. The Law of Mass Action requires that this rate be proportional to the product of concentrations involved in all reactions in which X plays a role. To better illustrate

how to translate a mechanism into rate laws, let us separate (2.1) into three steps:

$$(1)\ X + Z \overset{k_1}{\rightarrow} C$$

$$(2)\ X + Z \overset{k_2}{\leftarrow} C$$

$$(3)\ C \overset{k_3}{\rightarrow} Y + Z$$

Consider the effect each of (1), (2) and (3) has on $\frac{dx}{dt}$. Step (1) tells us that X is lost at a rate proportional to both X and Z. Therefore $\frac{dx}{dt}$ has a term of the form $-k_1xz$. Step (2) requires that we add a term of the form $+k_2c$. Step (3) tells us nothing about the concentration x so that the complete rate law for X is $\frac{dx}{dt} = -k_1xz + k_2c$. Rate laws for $\frac{dz}{dt}$, $\frac{dc}{dt}$ and $\frac{dy}{dt}$ can written similarly.

The equation $\frac{dx}{dt} = -k_1xz + k_2c$ has both first and second order terms. As before, we say that k_2c is a first order term because only one chemical concentration, c, is involved. However, $-k_1xz$ contains the product of two chemical concentrations and therefore represents a *second order* kinetic term. In general, the number of concentrations contained in a simple product determines the order of the kinetics. Rate laws including more complicated combinations of the chemical concentrations are also possible. The most important of these is the *Michaelis-Menten* rate law.

Let us define the *velocity* of the system described by (2.1) as the rate at which the product Y appears. We may express this velocity as $V = \frac{dy}{dt} = k_3c$. In general, we don't have an explicit solution for c so that we must consider the full set of differential equations derived from the Law of Mass Action. A careful consideration of these equations[3] will reveal an expression for V of the form

$$V = \frac{V_{max}x}{K_m + x} \tag{2.2}$$

where V_{max} represents a maximum velocity for the reaction defined as the product of k_3 and the total (constant) enzyme concentration. $K_m = \frac{k_2 + k_3}{k_1}$ is called the *Michaelis* constant. Equation (2.2) is called the *Michaelis-Menten equation* or *rate law* and is commonly used to describe an enzyme-catalyzed reaction.

[3]See [2], pp. 375-377.

3. Phase Plane Analysis of Biochemical Oscillations

We begin our mathematical investigation of biochemical oscillations in two dimensions by reviewing some facts from linear stability analysis. Consider the general two-variable system of ordinary differential equations

$$\begin{aligned} \dot{x} &= f(x,y) \\ \dot{y} &= g(x,y) \end{aligned} \tag{3.1}$$

where x and y are nonnegative quantities representing concentrations of the chemicals X and Y. This allows us to confine our analysis to the first quadrant of the phase plane. Solving

$$\begin{cases} f(x,y) = 0 \\ g(x,y) = 0 \end{cases} \tag{3.2}$$

will produce a steady state solution, (x_0, y_0), for the planar system (3.1). A chemical system generally possesses a stable steady state, but if stability is lost at a *Hopf bifurcation,* we are guaranteed that a periodic solution exists in a neighborhood of the steady state.[4] We will therefore look for oscillations generated at Hopf bifurcations.[5] The specific conditions for the Hopf bifurcation will be explained below.

We determine the stability of (x_0, y_0) by linearizing (3.1). The *Taylor expansion* about (x_0, y_0) has the general form

$$\begin{aligned} \dot{x} = \;& f(x_0,y_0) + f_x(x_0,y_0)(x-x_0) \\ & + f_y(x_0,y_0)(y-y_0) \\ & + O((x-x_0)^2, (y-y_0)^2) \\ \dot{y} = \;& g(x_0,y_0) + g_x(x_0,y_0)(x-x_0) \\ & + g_y(x_0,y_0)(y-y_0) \\ & + O((x-x_0)^2, (y-y_0)^2) \end{aligned} \tag{3.3}$$

[4] Confer [7], pp. 473-482.

[5] More complex bifurcations are possible, but will not be considered.

At the steady state (x_0, y_0), both $f(x_0, y_0)$ and $g(x_0, y_0)$ vanish. Making the substitution $\hat{x} = x - x_0$ and $\hat{y} = y - y_0$ and setting $\vec{u} = \begin{pmatrix} \hat{x} \\ \hat{y} \end{pmatrix}$, we may drop the nonlinear terms (provided $|\hat{x}| \ll 1$ and $|\hat{y}| \ll 1$) and rewrite system (3.3) as a linearized, planar system

$$\begin{aligned} \dot{\vec{u}} &= \begin{pmatrix} f_x(x_0, y_0) & f_y(x_0, y_0) \\ g_x(x_0, y_0) & g_y(x_0, y_0) \end{pmatrix} \vec{u} \\ &= \begin{pmatrix} \alpha & \beta \\ \gamma & \delta \end{pmatrix} \vec{u} \end{aligned} \tag{3.4}$$

The matrix $J_0 = \begin{pmatrix} \alpha & \beta \\ \gamma & \delta \end{pmatrix}$ is the called the *Jacobian matrix* of (3.1) at (x_0, y_0). Set $\tau = \text{trace}(J_0) = \alpha + \delta$ and $\Delta = \det(J_0) = \alpha\delta - \beta\gamma$. Then the eigenvalues, λ, of the Jacobian matrix, which satisfy $\det(J_0 - \lambda I_2) = 0$, can be used to determine the stability of (3.4). Since

$$\begin{aligned} \det(J_0 - \lambda I_2) &= \det \begin{pmatrix} \alpha - \lambda & \beta \\ \gamma & \delta - \lambda \end{pmatrix} \\ &= (\alpha - \lambda)(\delta - \lambda) - \beta\gamma \\ &= \lambda^2 - (\alpha + \delta)\lambda + \alpha\delta - \beta\gamma \\ &= \lambda^2 - \tau\lambda + \Delta \end{aligned}$$

the solutions for λ are

$$\lambda = \frac{\tau \pm \sqrt{\tau^2 - 4\Delta}}{2} \tag{3.5}$$

According to linear stability analysis, (x_0, y_0) is stable or unstable depending on whether the real part of λ is negative or positive, respectively. When $\Delta < 0$, the discriminant of (3.5) is positive so that λ is real. Further, $\sqrt{\tau^2 - 4\Delta} > |\tau|$, which implies that one solution is positive while the other is negative. Therefore, the steady state is always a saddle point, which is always unstable. On the other hand, when $\Delta > 0$, we must consider the sign of the discriminant in order to find $\Re(\lambda)$. If $\tau^2 \geq 4\Delta$, then λ is real and $\sqrt{\tau^2 - 4\Delta} < |\tau|$ implies that both solutions are of the same sign. In this case, (x_0, y_0) is always a *stable* or *unstable node.* If $\tau^2 < 4\Delta$, then a pair of complex conjugates satisfies (3.5). The real part of these solutions is τ. Thus, only in

this case is it possible for the real part of λ to change signs. That is, (x_0, y_0) changes stability if and only if the solutions for the eigenvalues of J_0 are a complex conjugate pair and the trace(J_0) changes sign.[6]

Set $\lambda = \mu \pm i\omega$. Then the solutions to the *linear system* (3.4) are of the form $ce^{\lambda t} = ce^{(\mu \pm i\omega)t} = ce^{\mu t}e^{\pm i\omega t} = ce^{\mu t}(\cos(\omega t) \pm \sin(\omega t))$. If $\Delta > 0$ with $\tau^2 < 4\Delta$, then ω is nonzero and there will be oscillatory solutions. Stability analysis guarantees that as long as the real part of the eigenvalues of linearized system are nonzero, then the stability of the original system (3.1) is determined by the linearized system. Graphically, if the solutions for λ cross through the imaginary axis in the complex plane anywhere other than the origin, the stability of (3.4), and hence (3.1), will change (see Figure 2) and we might expect to see oscillatory behavior. In fact, this is precisely what defines a *Hopf bifurcation.*

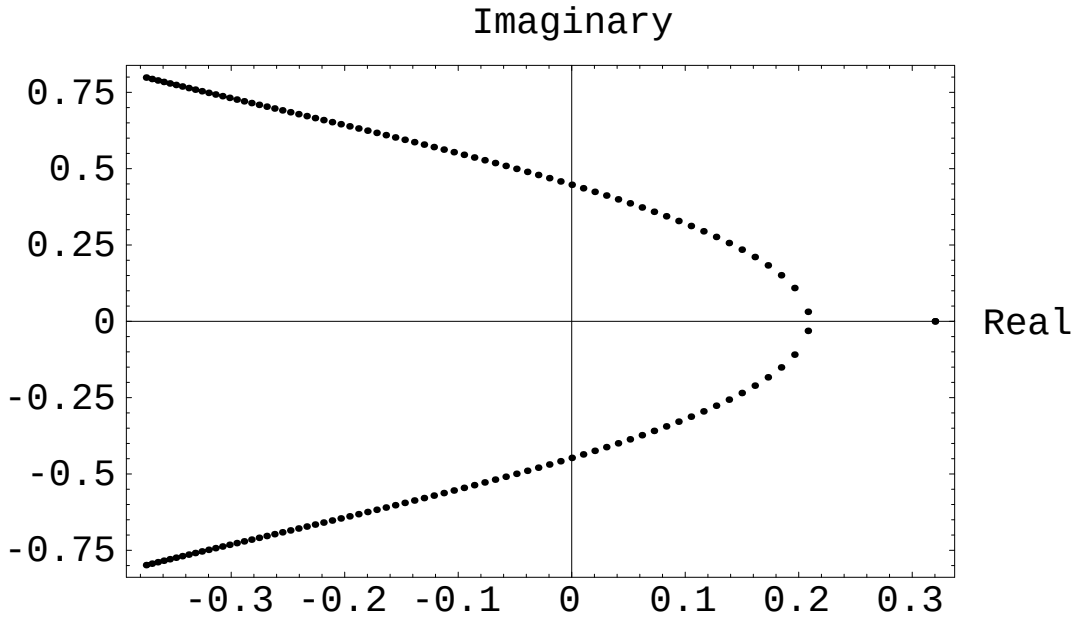

FIGURE 2. Eigenvalues Passing Through a Hopf Bifurcation

In terms of the solutions (3.5), Hopf bifurcations occur when $\tau = 0$ and $\Delta > 0$. That is, when $\alpha = -\delta$ and $\alpha\delta > \beta\gamma$. Since $\alpha\delta < 0$, it must be that β and γ are also of opposite sign. Without loss of generality,[7] we can assume that $\alpha > 0$ and $\delta < 0$ so

[6]The stability of the steady state solution in degenerate cases when $\Delta = 0$ or $\tau = 0$ must be treated on a case by case basis. See [8].

[7]... renaming the variables if necessary...

that only two possible sign patterns emerge.[8]

$$J_{SD} = \begin{pmatrix} + & + \\ - & - \end{pmatrix} \text{ and } J_{AI} = \begin{pmatrix} + & - \\ + & - \end{pmatrix}$$

Consideration of J_{SD}

Consider the linearized system (3.4) with a Jacobian matrix containing elements whose sign pattern is given by J_{SD}. We note the following conditions characterizing the system:

$$(1)\ f_x(x_0, y_0) > 0$$
$$(2)\ f_y(x_0, y_0) > 0$$
$$(3)\ g_x(x_0, y_0) < 0$$
$$(4)\ g_y(x_0, y_0) < 0$$

These conditions come directly from J_{SD} and are satisfied *at the steady state solution.* Condition (1) gives us that X reproduces itself by autocatalysis. Condition (2) and (3) together form a negative feedback loop[9] because $f_y(x_0, y_0)g_x(x_0, y_0) < 0$. Condition (4) simply represents degradation of Y and therefore plays no special role. See Figure 3 for a diagram representing such a system.

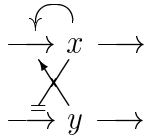

Figure 3. A Basic Diagram for a System Based on J_{SD} : X reproduces itself autocatalytically and negatively affects Y while Y positively affects X.

The oscillator characterized in this system is generally used to describe a two-component, chemical system called the *Substrate Depletion* oscillator. The chemical

[8]In fact, we have ignored when α or $\delta = 0$ and/or when β or $\gamma = 0$. These are limiting cases and add nothing to our discussion.

[9]See section 1 for a definition of negative feedback.

Y acts as fuel for a process whose endproduct is the chemical X. Further, X has a tendency to catalyze its own production, so a more common way to represent this system can be seen in Figure 4.

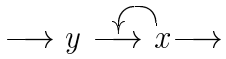

FIGURE 4. A Common Representation for Substrate Depletion Oscillator

The potential for the substrate depletion mechanism to cause oscillation is clear. Suppose the reaction begins with a large concentration of Y and a low concentration of X. The reaction may start slowly, but as X builds up, it catalyzes the reaction, eventually causing an overdepletion of the substrate Y. The process grinds to a halt because Y can no longer be converted into X. Then, the X-process is dormant until Y is able to replenish itself, at which point the process may begin anew.

CONSIDERATION OF J_{AI}

On the other hand, if the linearized system (3.4) produces a Jacobian matrix containing elements whose signs are determined by J_{AI}, we say the system is an *Activator-Inhibitor* oscillator. The conditions, deduced from J_{AI}, are

$$(1)\ f_x(x_0, y_0) > 0$$
$$(2)\ f_y(x_0, y_0) < 0$$
$$(3)\ g_x(x_0, y_0) > 0$$
$$(4)\ g_y(x_0, y_0) < 0$$

Again, condition (1) signifies autocatalysis while conditions (2) and (3) together form a negative feedback loop. However, in contrast to the substrate depletion system, the roles of x and y are reversed. Condition (4) again represents the degradation of Y and plays no special role. See Figure 5 for a diagrammatic representation of this system.

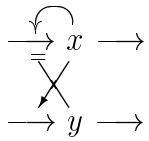

FIGURE 5. A Basic Diagram for a System Based on $J_{AI} : X$ reproduces itself autocatalytically and positively affects Y while Y negatively affects X.

That x and y reverse their roles as components of the negative feedback loop is chemically significant and changes our interpretation of the what mechanism is described by this model. We have the autocatalytic "activator" X producing a chemical Y. In turn, the "inhibitor" Y slows the X-process. A better representation of this system is given in Figure 6. The potential for the activator-inhibitor mechanism to

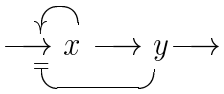

FIGURE 6. A Common Representation of an Activator-Inhibitor Oscillator

cause oscillation is also clear. This time, the reaction may begin with low concentrations of both X and Y. X begins to activate its own production and as X builds up, Y begins to inhibit the X-process. As X decays, Y is unable to sustain itself and decays as well. This gives X the opportunity to restart process because it is no longer inhibited by the presence of Y.

REQUIREMENTS FOR OSCILLATION

A two-component biochemical system will oscillate only when two requirements are met. The first requirement is for the autocatalytic production of one of the components. In two variables, this requirement is met when the main diagonal of the Jacobian matrix of the linearized system has a positive term. The second requirement is for negative feedback. This is satisfied when $f_y(x_0, y_0)g_x(x_0, y_0) < 0$. Neither autocatalysis nor negative feedback alone is capable of producing oscillation.

In the absence of autocatalysis, $f_x(x,y) + g_y(x,y) < 0$ everywhere in the first quadrant. *Bendixson's Criterion*[10] states that if $f_x(x,y) + g_y(x,y)$ is of constant sign and not identically zero in the first quadrant, then (3.1) has no periodic solutions. Therefore, one of $f_x(x,y)$ and $g_y(x,y)$ must be positive if we are to expect a periodic solution. We may therefore conclude that autocatalysis is a necessary condition for oscillation of a two-component chemical system.

Furthermore, let us assume without loss of generality that x is an autocatalytic variable so that $f_x(x,y) > 0$ and $g_y(x,y) < 0$. As discussed earlier, a Hopf bifurcation can only occur if the determinant of the Jacobian matrix is *positive.* But $\Delta = f_x(x,y)g_y(x,y) - g_x(x,y)f_y(x,y) > 0$ so that $f_y(x,y)$ and $g_x(x,y)$ must differ in sign. This is precisely the requirement that there be negative feedback. Therefore, both autocatalysis and negative feedback are necessary to the production of oscillation in a two component system.

4. Oscillation in Systems of Three or More Variables

We now consider more complicated systems of three or more variables. We concluded above that the general planar system exhibits oscillation only in the presence of both an autocatalytic variable and negative feedback. Let us consider positive and negative feedback alone as possible bases for oscillation in three variables. The general three-variable system is

$$\begin{aligned} \dot{x}_1 &= f_1(x_1, x_2, x_3) \\ \dot{x}_2 &= f_2(x_1, x_2, x_3) \\ \dot{x}_3 &= f_3(x_1, x_2, x_3) \end{aligned} \tag{4.1}$$

with x_1, x_2 and x_3 all positive quantities representing chemical concentrations. Solving

$$\begin{aligned} f_1(x_1, x_2, x_3) &= 0 \\ f_2(x_1, x_2, x_3) &= 0 \\ f_3(x_1, x_2, x_3) &= 0 \end{aligned}$$

produces a steady state solution $(\bar{x}_1, \bar{x}_2, \bar{x}_3)$. We may linearize with a Taylor expansion about the point $(\bar{x}_1, \bar{x}_2, \bar{x}_3)$. Isolating a simple feedback loop in which autocatalysis

[10]See [7], pp. 372-374.

plays no role in (4.1) produces a linearization of the form $\dot{\vec{u}} = J_{\pm}\vec{u}$, where

$$J_{\pm} = \begin{pmatrix} -\alpha & 0 & \pm\phi \\ c_1 & -\beta & 0 \\ 0 & c_2 & -\gamma \end{pmatrix}$$

is the Jacobian matrix evaluated at $(\bar{x}_1, \bar{x}_2, \bar{x}_3)$ and $\alpha, \beta, \gamma, c_1, c_2, \phi$ are all positive constants. The negative signs on the main diagonal assure us of the absence of an autocatalytic variable. J_+ represents pure positive feedback while J_- represents pure negative feedback.

J_+ and the Routh-Hurwitz Theorem

Consider

$$J_+ = \begin{pmatrix} -\alpha & 0 & \phi \\ c_1 & -\beta & 0 \\ 0 & c_2 & -\gamma \end{pmatrix}$$

To determine whether biochemical oscillations are possible in a system with pure positive feedback, we look for conditions for a Hopf bifurcation. The eigenvalues of J_+ satisfy the equation $\det(J_+ - \lambda I_3) = 0$. Set $a_1 = \alpha + \beta + \gamma$, $a_2 = \alpha\beta + \beta\gamma + \alpha\gamma$ and $a_3 = \alpha\beta\gamma - \phi c_1 c_2$. Since

$$\begin{aligned} \det \begin{pmatrix} -\alpha - \lambda & 0 & \phi \\ c_1 & -\beta - \lambda & 0 \\ 0 & c_2 & -\gamma - \lambda \end{pmatrix} &= -(\alpha + \lambda)(\beta + \lambda)(\gamma + \lambda) + \phi c_1 c_2 \\ &= \lambda^3 + (\alpha + \beta + \gamma)\lambda^2 \\ &\quad +(\alpha\beta + \beta\gamma + \alpha\gamma)\lambda \\ &\quad +\alpha\beta\gamma - \phi c_1 c_2 \\ &= \lambda^3 + a_1\lambda^2 + a_2\lambda + a_3 \end{aligned}$$

our eigenvalues satisfy the characteristic equation $\lambda^3 + a_1\lambda^2 + a_2\lambda + a_3 = 0$. Explicitly solving for the solutions of this equation is a difficult task. We make use of the *Routh-Hurwitz Theorem* to show that at most one eigenvalue is positive, i.e., that the unstable manifold is at most a one-dimensional subspace. As discussed earlier, a

Hopf bifurcation can only happen when the real part of a *pair* of complex conjugate eigenvalues becomes positive.

For a cubic polynomial, the Routh-Hurwitz Theorem states that if

$$\Delta_1 = a_1 > 0$$
$$\Delta_2 = \det \begin{pmatrix} a_1 & 1 \\ a_3 & a_2 \end{pmatrix} > 0$$
$$\Delta_3 = \det \begin{pmatrix} a_1 & 1 & 0 \\ a_3 & a_2 & a_1 \\ 0 & 0 & a_3 \end{pmatrix} > 0,$$

then $\Re(\lambda_i) < 0$ for $i = 1, 2, 3$. Clearly $\Delta_1 = a_1 = \alpha + \beta + \gamma > 0$. Further,

$$\begin{aligned} \Delta_2 &= \det \begin{pmatrix} a_1 & 1 \\ a_3 & a_2 \end{pmatrix} \\ &= a_1 a_2 - a_3 \\ &= (\alpha + \beta + \gamma)(\alpha\beta + \beta\gamma + \alpha\gamma) - \alpha\beta\gamma + \phi c_1 c_2 \\ &> 0 \end{aligned}$$

since $\alpha\beta\gamma < (\alpha + \beta + \gamma)(\alpha\beta + \beta\gamma + \alpha\gamma)$ for positive values of the constants. Now,

$$\begin{aligned} \Delta_3 &= \det \begin{pmatrix} a_1 & 1 & 0 \\ a_3 & a_2 & a_1 \\ 0 & 0 & a_3 \end{pmatrix} \\ &= a_1 a_2 a_3 - a_3^2 \\ &= a_3(a_1 a_2 - a_3) \\ &= a_3 \Delta_2 \end{aligned}$$

so that the sign of Δ_3 depends on the sign of $a_3 = \alpha\beta\gamma - \phi c_1 c_2$. That is, if $a_3 > 0$, then $\Re(\lambda_i) < 0$ for $i = 1, 2, 3$. This excludes the possibility of a Hopf bifurcation when $\alpha\beta\gamma > \phi c_1 c_2$.

To show that a Hopf bifurcation is impossible for $\alpha\beta\gamma < \phi c_1 c_2$, let $g(\lambda) = \lambda^3 + a_1\lambda^2 + a_2\lambda + a_3$ be the characteristic equation for J_+. Recalling Descartes' Rule of Signs, we can say that $g(\lambda)$ has one positive real root, λ_0, because both a_1 and a_2 are

positive while a_3 is negative. This gives only a single sign change in $g(\lambda)$, and hence, one positive real root. Let $h(\lambda)$ be the polynomial such that $g(\lambda) = (\lambda - \lambda_0)h(\lambda)$. We can then find a set of nonlinear equations for the coefficients of $h(\lambda)$ by solving

$$\begin{aligned}\lambda^3 + a_1\lambda^2 + a_2\lambda + a_3 &= (\lambda - \lambda_0)h(\lambda) \\ &= (\lambda - \lambda_0)(\lambda^2 + b_1\lambda + b_2) \\ &= \lambda^3 + (b_1 - \lambda_0)\lambda^2 + (b_2 - \lambda_0 b_1)\lambda - \lambda_0 b_2\end{aligned}$$

Clearly

$$\begin{aligned}b_1 - \lambda_0 &= a_1 > 0 \\ b_2 - b_1\lambda_0 &= a_2 > 0 \\ -b_2\lambda_0 &= a_3 < 0\end{aligned}$$

Thus, $b_1 = a_1 + \lambda_0 > 0, b_2 = a_2 + b_1\lambda_0 > 0$. We may apply the Routh-Hurwitz theorem to $h(\lambda)$: since $b_1 > 0$ and $b_1 b_2 > 0$ (i.e., Δ_1 and Δ_2 for $h(\lambda)$), the real part of λ is negative for the remaining two eigenvalues. Thus, there is no Hopf bifurcation.

J_-: The Negative Feedback Oscillator

Consider

$$J_- = \begin{pmatrix} -\alpha & 0 & -\phi \\ c_1 & -\beta & 0 \\ 0 & c_2 & -\gamma \end{pmatrix}$$

This Jacobian represents a three-variable system with pure negative feedback. The above analysis for J_+ relied on the Routh-Hurwitz determinants and it was found that, with $a_1 = \alpha + \beta + \gamma, a_2 = \alpha\beta + \beta\gamma + \alpha\gamma$ and $a_3 = \alpha\beta\gamma - \phi c_1 c_2$, no Hopf bifurcation could occur. We see that replacing ϕ by $-\phi$ guarantees that $a_3 > 0$ so that the sign of $\Delta_3 = a_3\Delta_2$ depends on $\Delta_2 = (\alpha+\beta+\gamma)(\alpha\beta+\beta\gamma+\alpha\gamma) - \alpha\beta\gamma - \phi c_1 c_2$ rather than on a_3. Let $\Gamma = (\alpha + \beta + \gamma)(\alpha\beta + \beta\gamma + \alpha\gamma) - \alpha\beta\gamma$. We note $\Gamma > 0$ so that if $\Delta_2 = \Gamma - \phi c_1 c_2 > 0$, we will have no Hopf bifurcation by the Routh-Hurwitz theorem. However, when $\Gamma < \phi c_1 c_2$, oscillation is quite possible.

5. Goodwin's Equations

In 1963, Brian C. Goodwin introduced a set of equations that became the quintessential example of a biochemical oscillator based on negative feedback.

The general n-variable system of equations is[11]

$$\begin{aligned}
\dot{x}_1 &= \frac{a}{1+x_n^\rho} - \alpha_1 x_1 \\
\dot{x}_2 &= c_1 x_1 - \alpha_2 x_2 \\
&\vdots \\
\dot{x}_n &= c_{n-1} x_{n-1} - \alpha_n x_n
\end{aligned}$$

To simplify, let us consider this system for $\alpha_1 = \alpha_2 = \cdots = \alpha_n$:

$$\begin{aligned}
\dot{x}_1 &= \frac{a}{1+x_n^\rho} - \alpha x_1 \\
\dot{x}_2 &= c_1 x_1 - \alpha x_2 \\
&\vdots \\
\dot{x}_n &= c_{n-1} x_{n-1} - \alpha x_n .
\end{aligned} \tag{5.1}$$

Since it will become important to us later, let us solve for the steady state solution $(\bar{x}_1, \ldots, \bar{x}_n)$. By setting each of equation of (5.1) equal to zero, we find that

$$\begin{aligned}
\frac{a}{1+\bar{x}_n^\rho} &= \alpha \bar{x}_1 \\
&= \frac{\alpha^2}{c_1} \bar{x}_2 \\
&\vdots \\
&= \frac{\alpha^n}{c_1 \ldots c_{n-1}} \bar{x}_n
\end{aligned}$$

Setting $\beta^n = a c_1 \ldots c_{n-1}$ and $\delta = \dfrac{\alpha}{\beta}$ allows us to write the steady state equation as

$$\frac{1}{1+\bar{x}_n^\rho} = \delta^n \bar{x}_n \tag{5.2}$$

[11] See MacDonald [5].

or

$$\delta^n \bar{x}_n^{\rho+1} = 1 - \delta^n \bar{x}_n. \tag{5.3}$$

Descartes' Rule of Signs guarantees a unique positive solution, $\bar{x}_n > 0$, to this equation.

We may then linearize the system using Taylor expansions about the steady state. The resulting system has the Jacobian matrix

$$J = \begin{pmatrix} -\alpha & 0 & \dots & 0 & -\phi^n a \\ c_1 & -\alpha & 0 & \dots & 0 \\ 0 & c_2 & -\alpha & \dots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & \dots & 0 & c_{n-1} & -\alpha \end{pmatrix}$$

where $\phi^n = \dfrac{\rho \bar{x}_n^{\rho-1}}{(1+\bar{x}_n^{\rho})^2}$. Solving $\det(J - \lambda I_n) = 0$ produces the characteristic equation

$$(-1)^n(\alpha+\lambda)^n - (-1)^{n+1}\phi^n a c_1 \dots c_{n-1} = 0$$

and since $ac_1c_2 \dots c_{n-1} = \beta^n$, this simplifies to

$$(\alpha+\lambda)^n + \phi^n \beta^n = 0 \tag{5.4}$$

The choice for equal α's is now clear: without this assumption, solving this characteristic equation analytically would be impossible. As it stands, we easily conclude that $\lambda = (-1)^{\frac{1}{n}}\phi\beta - \alpha$. At a Hopf bifurcation, it must be that $\Re(\lambda) = \phi\beta\cos(\frac{(2k+1)\pi}{n}) - \alpha = 0$ for some integer k. This condition is first satisfied for $k = 0$. If $k = 0$, then $\Re(\lambda) = \phi\beta\cos(\frac{\pi}{n}) - \alpha$. For the steady state to be unstable, we must

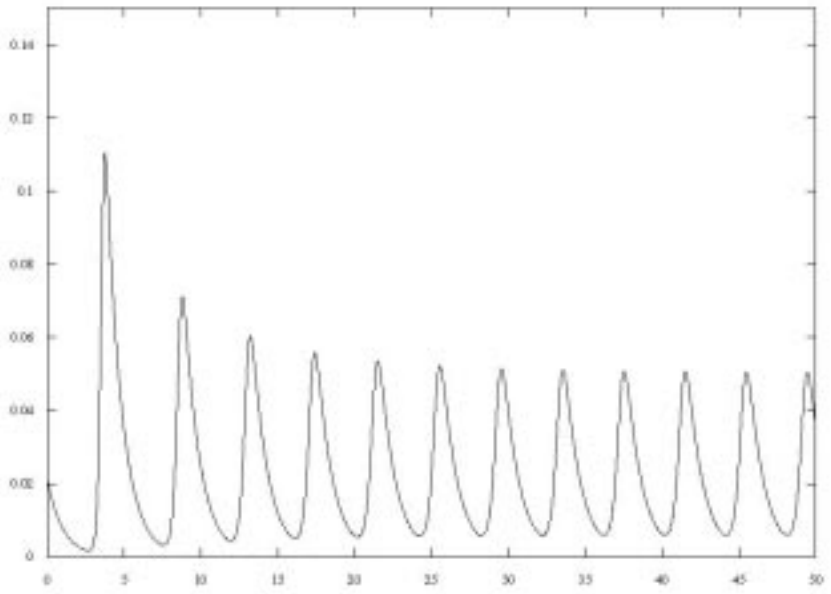

FIGURE 7. Oscillation in Goodwin's Model: An oscillatory solution to Goodwin's equations for $a = c_1 = c_2 = \cdots = c_{n-1} = 1$, $n = 3$, $\rho = 10$ and $\alpha = 0.1$. In this case, ρ is large enough that the system may oscillate.

have $\phi > \delta\gamma$, where $\gamma = \dfrac{1}{\cos(\frac{\pi}{n})}$. Using equations (5.2) and (5.3), we find that

$$\begin{aligned}
\phi^n &= \frac{\rho \bar{x}_n^{\rho-1}}{(1+\bar{x}_n^{\rho})^2} \\
&= \rho \bar{x}_n^{\rho-1} (\frac{1}{1+\bar{x}_n^{\rho}})^2 \\
&= \rho \bar{x}_n^{\rho-1} \delta^{2n} \bar{x}_n^2 \\
&= \rho \bar{x}_n^{\rho+1} \delta^{2n} \\
&= \rho \delta^n (\delta^n \bar{x}_n^{\rho+1}) \\
&= \rho \delta^n (1 - \delta^n \bar{x}_n)
\end{aligned}$$

Thus, to have an unstable steady state, it is sufficient that $\phi^n = \rho\delta^n(1-\delta^n\bar{x}_n) > \delta^n\gamma^n$. Thus, $\rho > \dfrac{\gamma^n}{1-\delta^n\bar{x}_n}$. For $n = 3$, $\rho > \dfrac{8}{1-\delta^3\bar{x}_3} > 8$ because $0 < \delta^3\bar{x}_3 =$

$\frac{1}{1+\bar{x}_3^{\rho}} < 1$. This result, namely that $\rho > 8$, was first reached by Griffith in 1968 [3]. Unfortunately, this requirement on ρ is biologically improbable. ρ is generally called

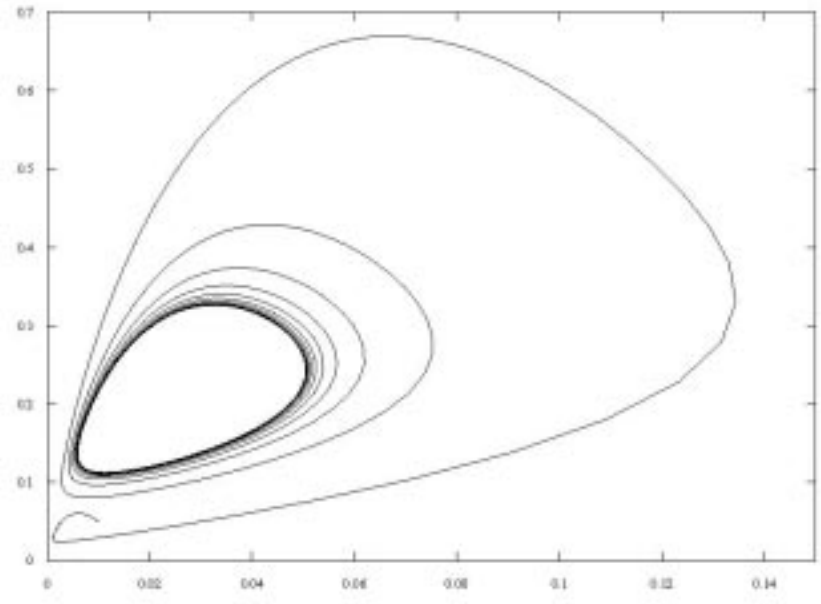

FIGURE 8. A Limit Cycle in Goodwin's Equations: Parameters are as in Figure 7. The solution is projected onto (x_1, x_2) plane.

the *Hill coefficient,* "the significance of which is that ρ molecules of the endproduct $[x_n]$ combine with an enzyme which catalyses the formation of the first chemical species in the chain to form an inactive substance" as explained by MacDonald [5]. Such a high order of *cooperative* repression is unlikely.[12] Thus, while Goodwin's equations may describe a basic mechanism for oscillation in a biochemical system of three or more variables, we would like to find some way to lower this constraint on ρ. This was done by Bliss and Painter [4], and is the topic of the next section.

Figures 7, 8 and 9 give typical solutions to Goodwin's equations. Figure 7 plots a solution in the (t, x_3) plane when $n = 3$, $\rho = 10$ and $\alpha = 0.1$. In this case, ρ is large enough so that the existence of a stable periodic solution is evident. Figure 8 reveals the approach of this solution to the limit cycle (i.e., periodic solution) in the

[12]See [4], pp. 177-178.

(x_1, x_2) phase space. When ρ is decreased to 5, the steady state becomes stable and the solution decays to this equilibrium (see figure 9).

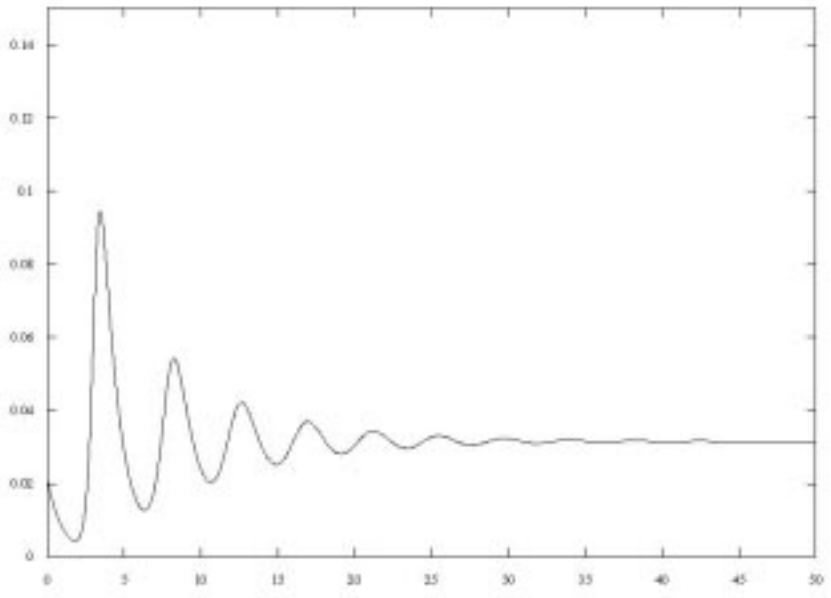

FIGURE 9. Stability: Parameters are as in Figure 7, but with $\rho = 5$. The decrease in ρ causes the steady state to become stable.

6. Painter's Modification of Goodwin's Equations

In an effort to reduce the constraint found above on ρ, Bliss and Painter [4] add a nonlinear term saying this is "appropriate when the endproduct is consumed in an enzyme-catalyzed reaction described by *Michaelis-Menten* kinetics." We therefore replace the linear degradation of the last component with a Michaelis-Menten term of the form $\dfrac{Vz}{J+z}$ to get

$$\begin{aligned} \dot{x} &= \frac{a}{1+z} - \alpha x \\ \dot{y} &= \alpha x - \alpha y \\ \dot{z} &= \alpha y - \frac{Vz}{J+z} \end{aligned} \tag{6.1}$$

where $a, \alpha, V, J > 0$ are constants. Admittedly, some generality is lost in assuming symmetry in the degradation of X and Y, but no generality is lost in writing the synthesis terms for Y and Z as αx and αy.[13]

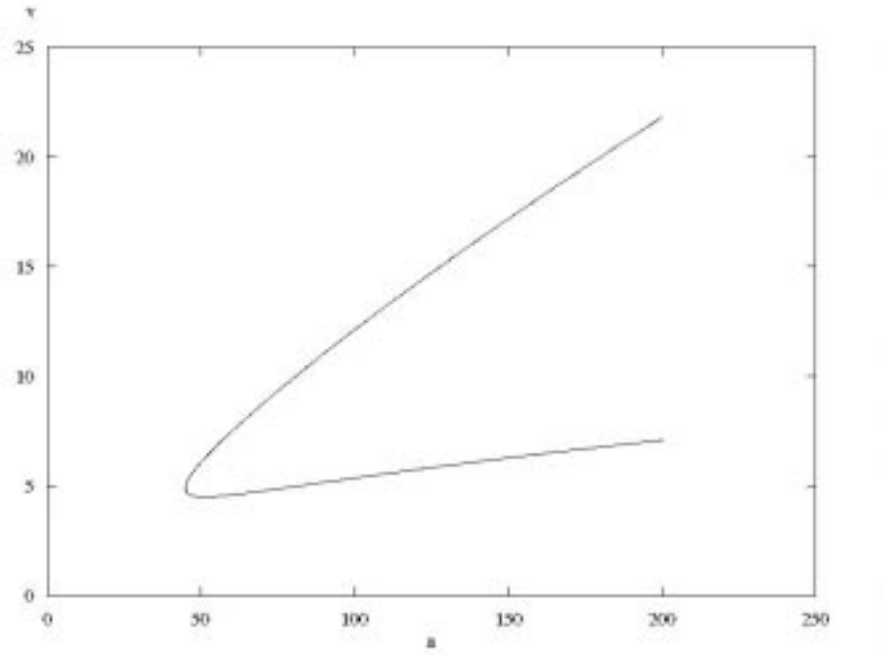

FIGURE 10. Locus of Hopf Bifurcations: For a fixed α (in this case $\alpha = 0.1$), we may compute a locus of bifurcation values in the (a, V) parameter plane. Note that the oscillatory domain lies "inside" the curve.

Again, it will be useful to have explicit solutions for the steady state.

$$\begin{aligned} \frac{a}{1+\bar{z}} &= \alpha \bar{x} \\ &= \alpha \bar{y} \\ &= \frac{V\bar{z}}{J+\bar{z}} \end{aligned}$$

[13]To see this, consider $\dot{y} = c_1\hat{x} - \alpha y$. Simply by setting $x = \frac{c_1}{\alpha}\hat{x}$, we immediately conclude that $\dot{y} = \alpha x - \alpha y$. y may be similarly scaled to ensure that the rate of synthesis of Z is αy.

If $J = 1$, $\bar{z} = \dfrac{a}{V}$. Then, linearizing the system at the steady state produces

$$J = \begin{pmatrix} -\alpha & 0 & -\phi \\ \alpha & -\alpha & 0 \\ 0 & \alpha & -\gamma \end{pmatrix}$$

as the Jacobian matrix, where $\phi = \dfrac{a}{(1+\bar{z})^2}$ and $\gamma = \dfrac{JV}{(J+\bar{z})^2}$. Again, the characteristic equation

$$(\gamma + \lambda)(\alpha + \lambda)^2 + \alpha^2\phi = 0 \tag{6.2}$$

is difficult to solve. We may simplify by supposing that $\gamma = \alpha$. In this simple case, we can easily find a Hopf bifurcation. Naturally, once we find a Hopf bifurcation, we may use it as a starting point to find other Hopf bifurcations with numerical tools.

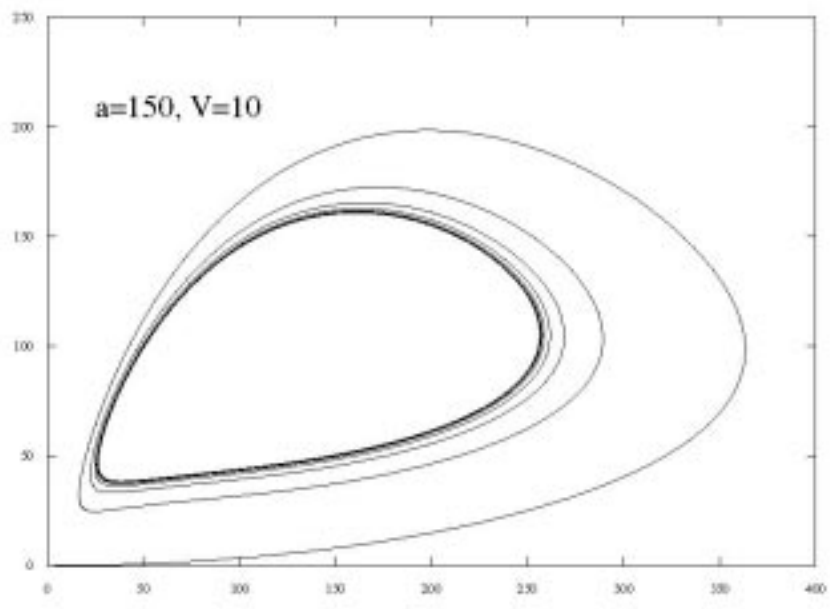

FIGURE 11. A Limit Cycle in Painter's Equations: Taking the point $(a, V) = (150, 10)$ from inside the oscillatory domain in Figure 10 (with $\alpha = 0.1$), we see a solution approaching the limit cycle in the (x_1, x_2) projection of phase space.

Let us choose a and V so that $\alpha = \gamma = \dfrac{JV}{(J+\bar{z})^2}$. When $J = 1$, $\bar{z} = \dfrac{a}{V}$ so that $\alpha = \dfrac{V}{(1+\frac{a}{V})^2} = \dfrac{V^3}{(V+a)^2}$. Choosing a and V properly assures us that $\alpha = \gamma$. Further, $\phi = \dfrac{a}{(1+\frac{a}{V})^2} = \dfrac{aV^2}{(V+a)^2} = \alpha\dfrac{a}{V}$. Let us solve equation (6.2). Replacing γ with α we find that

$$\begin{aligned}(\alpha+\lambda)^3 &= -\alpha^2\phi \\ &= -\alpha^3\frac{a}{V}\end{aligned}$$

Taking the cube root of both sides produces $\lambda = (-1)^{\frac{1}{3}}(\alpha^3\dfrac{a}{V})^{\frac{1}{3}} - \alpha$. Consider $\Re(\lambda) = \dfrac{1}{2}(\alpha^3\dfrac{a}{V})^{\frac{1}{3}} - \alpha$. For this to be positive we must have that $\dfrac{a}{V} > 8$. Thus, we have a complex conjugate pair of eigenvalues whose real part is positive for $\dfrac{a}{V} > 8$. This gives us a Hopf bifurcation.

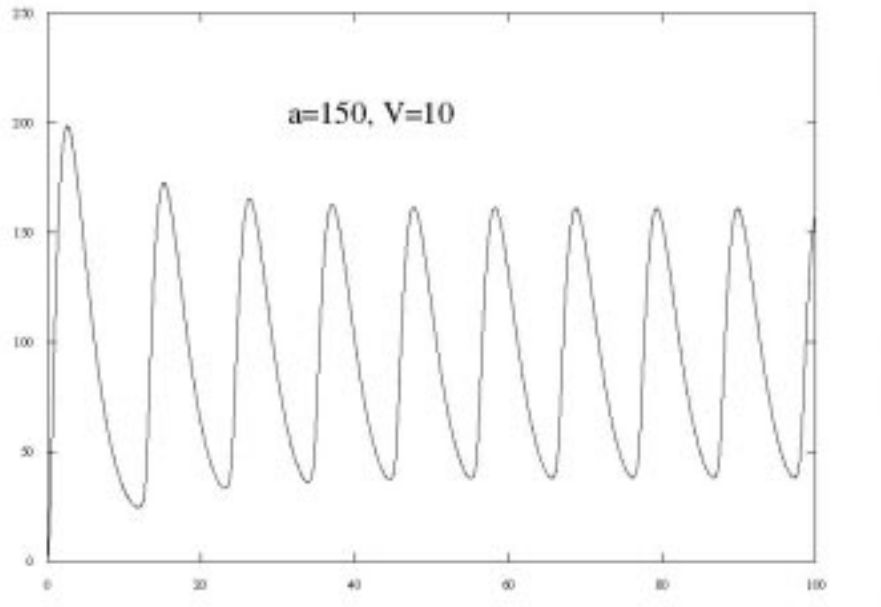

FIGURE 12. An Oscillatory Solution: The solution of Figure 11 plotted in the (t, x_2) plane.

To summarize, if $J = 1$ we can choose a and V so that $\gamma = \alpha$. Under these conditions, Bliss and Painter's generalization (6.1) of Goodwin's equations experiences a Hopf bifurcation when $\frac{a}{V} = 8$. This condition is equivalent to $\frac{V}{\alpha} = (1 + \frac{a}{V})^2 = 81$. For fixed α, this expression gives us a single point from a *locus* of Hopf bifurcations in (a, V) parameter space. We may then use numerical techniques[14] to generate the entire locus of Hopf bifurcations. Figure 10 shows this for $\alpha = 0.1$. The oscillatory domain lies "inside" or above the curve. Taking the point $(a, V) = (150, 10)$ inside the oscillatory domain, we compute a solution to (6.1) approaching the limit cycle. Figure 11 plots this approach to the limit cycle in the (x_1, x_2) plane while Figure 12 plots a solution in the (t, x_2) plane.

7. Distributed Time Lag and the Linear Chain Trick

As with the last section, the following analysis, due to N. MacDonald [5], reduces the constraint on the Hill coefficient in Goodwin's equations. In place of adding another non-linear term to the system, we introduce time delay. Goodwin's equations in three variables takes the form

$$\begin{aligned} \dot{x} &= \frac{\alpha_1}{1 + z^\rho} - \beta_1 x \\ \dot{y} &= \alpha_2 x - \beta_2 y \\ \dot{z} &= \alpha_3 y - \beta_3 z \end{aligned} \tag{7.1}$$

where $\alpha_i, \beta_i > 0$ are constants. Replacing the rate equation for Y with an integro-differential equation of the form

$$\dot{y} = \alpha_2 \int_{-\infty}^{t} x(\tau) G_a^p(t - \tau) d\tau - \beta_2 y \tag{7.2}$$

introduces a time delay on the production of Y based on the history of the chemical X. The rest of our discussion on distributed time lag will focus on equation (7.2) alone.

Distributed time lag refers specifically to the form of (7.2). Y's dependency on X is characterized by the *kernel function*, $G_a^p(s)$, defined by $G_a^p(s) = \frac{a^{p+1} s^p}{p!} e^{-as}$. The shape of this kernel is significant in that there is a peak at some time τ, a point in

[14] This was done using *xpp*, a unix-based, differential equations solver

the past around which we concentrate the dependency on X. Let us find the peak. Differentiating $G_a^p(s)$, we conclude that

$$\begin{aligned} \frac{d}{ds}G_a^p(s) &= \frac{a^{p+1}ps^{(p-1)}}{p!}e^{-as} - a\frac{a^{p+1}s^p}{p!}e^{-as} \\ &= a(\frac{a^p s^{(p-1)}}{(p-1)!}e^{-as} - \frac{a^{p+1}s^p}{p!}e^{-as}) \\ &= a(G_a^{p-1}(s) - G_a^p(s)) \end{aligned} \tag{7.3}$$

Solving $\frac{d}{ds}G_a^p(s) = 0$ tells us where the function will have its maximum value. It's a simple matter to check that $s = \frac{p}{a}$ solves the equation $G_a^{p-1}(s) = G_a^p(s)$ and tells us when we reach this maximum value. Further, we make the note that $\int_0^\infty G_a^p(s)ds = 1$. Figure 13 plots a typical example of the kernel function for $p = 4, a = 2$.

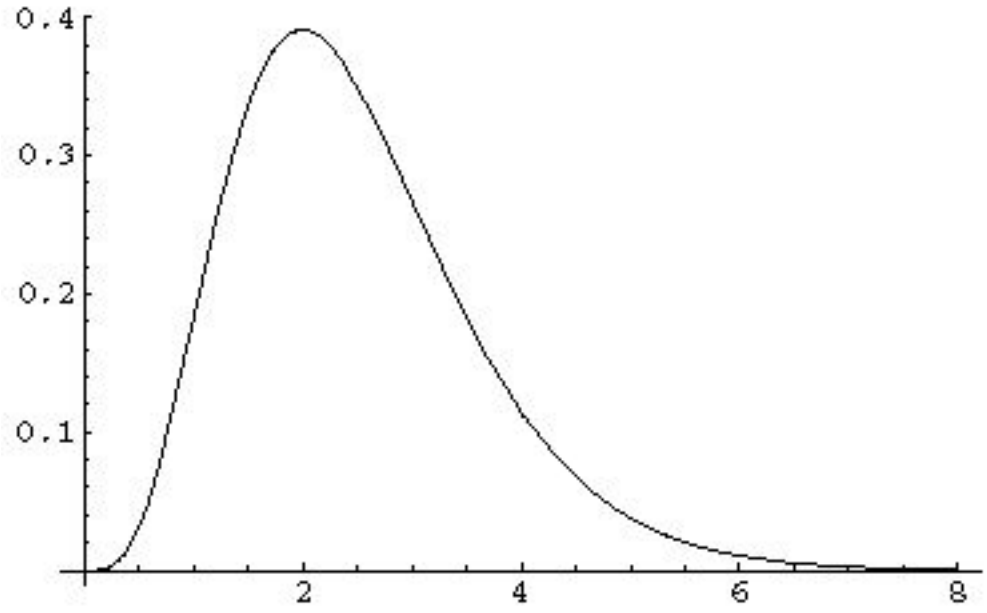

FIGURE 13. A typical kernel function, $G_a^p(s)$

Let us take a closer look at what was accomplished with equation (7.3). By choosing p and a, we control where the time dependency is to be concentrated. As p increases for a fixed value of the peak, the kernel becomes increasingly concentrated at a single value. *Discrete* time lag, therefore, can be thought of as an approximation

to *distributed* time lag where the discrete amount of delay is approximately equivalent to $\frac{p}{a}$, the peak of the kernel. More interestingly, equation (7.3) tells us that $G_a^p(s)$ satisfies a first order, *linear* differential equation. The *Linear Chain Trick* is based on this observation.

We proceed by defining

$$y_p(t) = \int_{-\infty}^{t} x(\tau) G_a^p(t-\tau) d\tau \tag{7.4}$$

Then, differentiating with respect to t, we find that

$$\begin{aligned} \frac{d}{dt} y_p(t) &= \frac{d}{dt} \int_{-\infty}^{t} x(\tau) G_a^p(t-\tau) d\tau \\ &= x(t) G_a^p(0) + \int_{-\infty}^{t} \frac{\partial}{\partial t} x(\tau) G_a^p(t-\tau) d\tau \end{aligned}$$

But $G_a^p(0) = 0$ so that, by utilizing (7.3), we have

$$\begin{aligned} \int_{-\infty}^{t} \frac{\partial}{\partial t} x(\tau) G_a^p(t-\tau) d\tau &= \int_{-\infty}^{t} x(\tau) \frac{\partial}{\partial t} G_a^p(t-\tau) d\tau \\ &= \int_{-\infty}^{t} x(\tau) (a(G_a^{p-1}(t-\tau) - G_a^p(t-\tau))) \\ &= a(\int_{-\infty}^{t} x(\tau) G_a^{p-1}(t-\tau) d\tau - \int_{-\infty}^{t} x(\tau) G_a^p(t-\tau) d\tau) \\ &= a(y_{p-1}(t) - y_p(t)) \end{aligned}$$

That is, $\dot{y}_p = a(y_{p-1} - y_p)$. Similarly,

$$\begin{aligned} \dot{y}_{p-1} &= a(y_{p-2} - y_{p-1}) \\ &\vdots \\ \dot{y}_1 &= a(y_0 - y_1) \end{aligned}$$

We complete the *linear chain* of differential equations by differentiating $y_0(t)$. Using the fact that $G_a^0(s) = ae^{-as}$ gives us the following.

$$\begin{aligned}\frac{d}{dt}y_0(t) &= \frac{d}{dt}a\int_{-\infty}^{t} x(\tau)e^{-a(t-\tau)}d\tau \\ &= a(x(t) - a\int_{-\infty}^{t} x(\tau)e^{-a(t-\tau)}d\tau \\ &= a(x(t) - y_0(t))\end{aligned}$$

Thus, based on the fact that the kernel satisfies a linear differential equation, we can reduce equation (7.2) to a chain of $p+2$ linear, first-order differential equations. Placing this chain back into the original system gives a new system of $3+p+1$ equations:

$$\begin{aligned}\dot{x} &= \frac{\alpha_1}{1+z^\rho} - \beta_1 x \\ \dot{y_0} &= ax - ay_0 \\ \dot{y_1} &= ay_0 - ay_1 \\ &\vdots \\ \dot{y_p} &= ay_{p-1} - ay_p \\ \dot{y} &= \alpha_2 y_p - \beta_2 y \\ \dot{z} &= \alpha_3 y - \beta_3 z\end{aligned}$$

This is just Goodwin's model with more components in the negative feedback loop.

8. Discrete Time Lag in a Minimal Model of the Circadian Rhythm

We now discuss a two-variable system of differential equations which recently appeared in an article of the *Journal of Neuroscience* [10]. The system is based on negative feedback and *discrete* time lag. We concluded earlier that a two-variable system based purely on negative feedback is incapable of producing oscillation. However, introducing discrete delay into such a system is sufficient to produce spontaneous oscillation. In section 6, we concluded that distributed delay simply increases the number of variables. Due to the fact that *discrete* delay approximates distributed delay, we can see that the addition of time lag, either discrete or distributed, has the same effect as adding variables to the system.

The model, as given by Scheper et al. [10], is

$$\begin{aligned} \dot{x} &= \frac{a}{1+y^n} - bx \\ \dot{y} &= cx(t-\tau)^m - dy \end{aligned} \tag{8.1}$$

where $m, n \in \mathbb{Z}^+$ and a, b, c, d and τ are positive constants. The variable x represents mRNA (*messenger RNA*) and the variable y represents the protein that is coded for by x. The delayed variable x appears in the synthesis term for y and depends on τ, the parameter defining the amount of delay. Thus, y is dependent on the concentration x at some point in the past. The exponent m is described as the "nonlinearity in the protein production cascade" while "n is the *Hill coefficient.*" The exponent n is comparable to ρ in Goodwin's equations. It therefore characterizes the cooperativity of the binding process between y and its mRNA. The constants a, b, c and d are rate constants associated with the chemical components of the system.[15]

Because "delay equations are notoriously difficult to solve analytically," Scheper et al. analyze (8.1) numerically. In contrast, we proceed analytically and limit ourselves to searching for Hopf bifurcations. This can be accomplished by assuming solutions of the form $ce^{\lambda t}$ for the linear system representing (8.1). We may then use linear stability analysis to determine conditions for a Hopf bifurcation. We are assured this system oscillates by the numerical results of Scheper et al.

[15]For a more complete description, see Scheper et al. [10].

We first find a steady state solution for (8.1). Solving for y_0 in the second equation gives us $y_0 = \frac{c}{d}x_0^m$. Substituting this expression into the first equation, we find that

$$b(\frac{c}{d})^n x_0^{mn+1} + bx_0 - a = 0 \tag{8.2}$$

The solution to this equation will give an explicit value for the steady state about which we may linearize. In general, this is a nontrivial computation and cannot be done analytically in any useful way. We shall see that further simplification is possible. Assuming that we have a solution to (8.2), we may linearize to get the system

$$\begin{aligned} \dot{u}(t) &= -bu - \beta v \\ \dot{v}(t) &= \gamma u_\tau - dv \end{aligned} \tag{8.3}$$

where the subscript τ denotes the dependency of u on the time lag (that is, $u_\tau = u(t-\tau)$) and β and γ are constants defined as follows:

$$\begin{aligned} \beta &= \frac{an{y_0}^{n-1}}{1+{y_0}^n} \\ &= \frac{an(\frac{c}{d}x_0^m)^{n-1}}{1+(\frac{c}{d}x_0^m)^n} \\ \gamma &= cm{x_0}^{m-1} \end{aligned}$$

parameter	fixed value
a	1
b	0.21
c	1
d	0.21
m	3
n	2
τ	4

FIGURE 14. Realistic Parameter Values

Rearranging (8.2), we find that $x_0^{mn} = \dfrac{(\frac{c}{d})^{-n}(a - bx_0)}{bx_0}$. Then $\beta\gamma$ can be simplified to give

$$\begin{aligned}\beta\gamma &= \frac{an(\frac{c}{d}x_0^m)^{n-1}}{1 + (\frac{c}{d}x_0^m)^n} cmx_0^{m-1} \\ &= \frac{admn(\frac{c}{d}x_0^m)^n}{x_0(1 + (\frac{c}{d}x_0^m)^n)^2} \\ &= bdmn(1 - \frac{b}{a}x_0)\end{aligned}$$

Let us assume solutions for (8.3) of the form $u(t) = \hat{u}e^{\lambda t}$ and $v(t) = \hat{v}e^{\lambda t}$, where $\hat{u}$ and $\hat{v}$ are the initial values. Substitute these into (8.3) to get the vector equation

$$\lambda \begin{pmatrix} \hat{u}e^{\lambda t} \\ \hat{v}e^{\lambda t} \end{pmatrix} = \begin{pmatrix} -b & -\beta \\ \gamma e^{-\lambda\tau} & -d \end{pmatrix} \begin{pmatrix} \hat{u}e^{\lambda t} \\ \hat{v}e^{\lambda t} \end{pmatrix}$$

Canceling out the $e^{\lambda t}$ on each side simplifies this to the standard eigenvalue equation

$$\lambda \begin{pmatrix} \hat{u} \\ \hat{v} \end{pmatrix} = \begin{pmatrix} -b & -\beta \\ \gamma e^{-\lambda\tau} & -d \end{pmatrix} \begin{pmatrix} \hat{u} \\ \hat{v} \end{pmatrix}$$

Thus, the characteristic equation is transcendental: $(b + \lambda)(d + \lambda) + \beta\gamma e^{-\lambda\tau} = 0$. Expanding, we find that

$$\lambda^2 + (b + d)\lambda + bd + \beta\gamma e^{-\lambda\tau} = 0$$

Again, the characteristic equation is impossible to solve analytically. To find a Hopf bifurcation, assume $\lambda = \mu \pm i\omega$. Substitute this into the characteristic equation, expanding and separating the resulting expression into the real and imaginary pieces reveals

$$\begin{aligned}\mu^2 - \omega^2 + (b + d)\mu + bd + \beta\gamma e^{-\mu\tau}\cos(\omega\tau) = 0 \\ 2\omega\mu + (b + d)\omega - \beta\gamma e^{-\mu\tau}\sin(\omega\tau) = 0\end{aligned}$$

because $\Re(e^{\mp i\omega\tau}) = \cos(\mp\omega\tau) = \cos(\omega\tau)$ while $\Im(e^{\mp i\omega\tau}) = \pm\sin(\mp\omega\tau) = -\sin(\omega\tau)$. At a Hopf bifurcation, $\mu = 0$ and $\omega \neq 0$ so that

$$\begin{aligned}\cos(\omega\tau) &= \frac{\omega^2 - bd}{\beta\gamma} \\ \sin(\omega\tau) &= \frac{(b + d)\omega}{\beta\gamma}\end{aligned}$$

These two conditions can be nondimensionalized[16] to give the simplified form

$$\begin{aligned} \cos(\omega\tau) &= \frac{\omega^2 - 1}{mn(1-\xi)} \\ \sin(\omega\tau) &= \frac{\rho\omega}{mn(1-\xi)} \end{aligned} \tag{8.4}$$

with $\rho = \dfrac{b+d}{\sqrt{bd}}$ and $\xi = \dfrac{b}{a}x$ satisfying the equation $(\dfrac{a}{b})^{mn}(\dfrac{c}{d})^{n}\xi^{mn+1} + \xi - 1 = 0$. This condition on ξ is obtained from (8.2) with x rescaled by $\dfrac{b}{a}$. Notice[17] that $\dfrac{\rho}{2} = \dfrac{\frac{b+d}{2}}{\sqrt{bd}} > 1$ so that $\rho > 2$. Then, to find ω,

$$\begin{aligned} (\frac{\omega^2 - 1}{mn(1-\xi)})^2 + (\frac{\rho\omega}{mn(1-\xi)})^2 &= \cos^2(\omega\tau) + \sin^2(\omega\tau) \\ &= 1 \end{aligned}$$

Expanding and simplifying, we see that

$$\omega^2 = \frac{-(\rho^2-2) \pm \sqrt{-(\rho^2-2)^2 + 4(m^2n^2(1-\xi)^2 - 1)}}{2}$$

For ω to be real, we must choose the additive solution. Without loss of generality, we may choose the positive solution for ω so that

$$\omega = \sqrt{\frac{-(\rho^2-2) \pm \sqrt{-(\rho^2-2)^2 + 4(m^2n^2(1-\xi)^2 - 1)}}{2}}$$

Now, combining the equations of (8.4), we see that

$$\tau = \frac{\tan^{-1}(\frac{\omega^2-1}{\rho\omega})}{\omega}$$

Using the above solution for ω, we may visualize the bifurcation behavior by plotting τ vs. other parameters of the system. Figure 15 plots a bifurcation curve in the (τ, a)

[16] This can be achieved by scaling ω by $\dfrac{1}{\sqrt{bd}}$.

[17] Note: $(b-d)^2 > 0 \longrightarrow (b+d)^2 > 4bd \longrightarrow \dfrac{\frac{b+d}{2}}{\sqrt{bd}} > 1$.

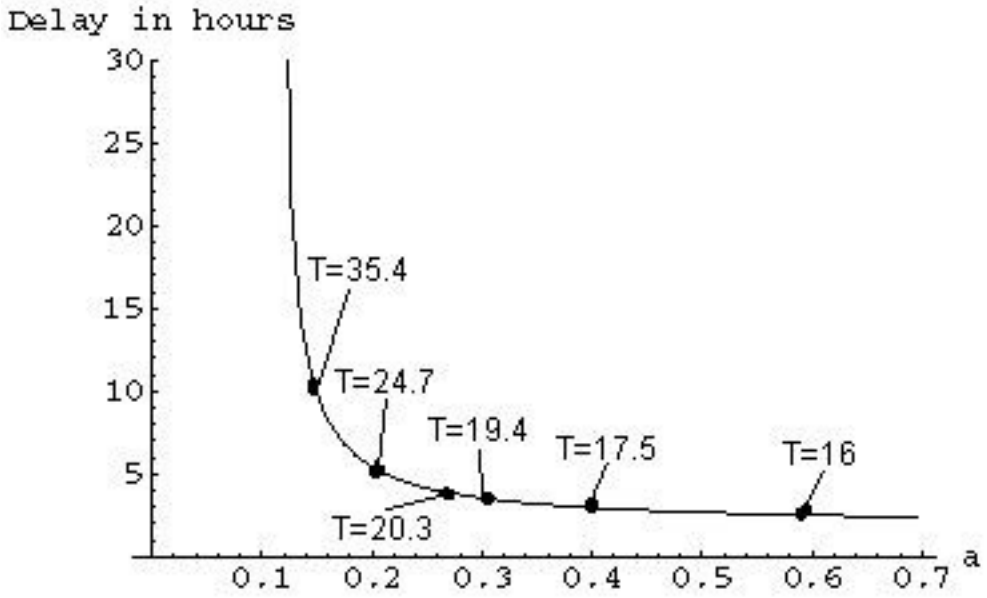

FIGURE 15. Bifurcation Curve in the (a, τ) plane: Fixed values of all other parameters are given in Figure 14. As a, the maximum velocity for synthesis of messenger RNA, is reduced, the larger the time delay necessary to produce oscillation. Various values for the period of oscillation at the bifurcation are indicated along the curve

plane based on this relationship.[18] Note that when $a = .27$, $\tau \approx 4$ which agrees with Scheper's values.

The (m, n, τ) subspace is interesting as well and verifies intuition: increased nonlinearity in the system, both in m and n, decreases the lag necessary to generate oscillation. Figure 16 is a plot of the bifurcation surface in this space. Note the gap in the graph at $(m, n) = (1, 1)$. This signifies that there is no possible oscillation in the linear case. This agrees with the analysis of Goodwin's equations; no matter the number of variables, the Hill coefficient must exceed 1 for oscillatory solutions.

To conclude, let us verify the period calculations at the Hopf bifurcations. From the data given by Scheper et al, we can construct the table seen in Figure 17. The first column tells us which parameter is allowed to vary while the second column gives the value at which (8.1) experiences a Hopf bifurcation. The third column gives the period

[18]In order to compare our results with those of Scheper et al, we have actually plotted τ in hours. That is, we have scaled by $\sqrt{bd}$. Also, we choose fixed parameters from Figure 14. Scheper et al. [10] give a detailed explanation of why these values are chosen.

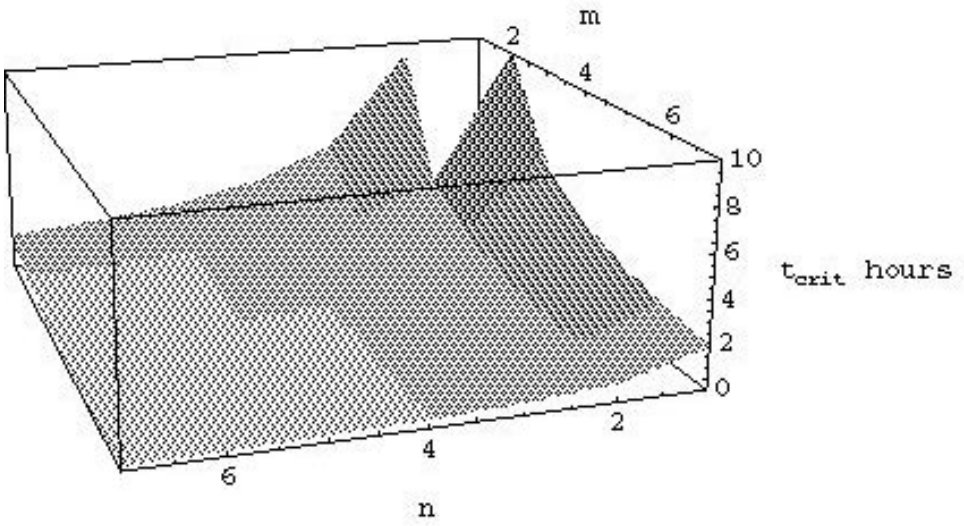

FIGURE 16. Bifurcation Surface for τ vs. m and n: We can see that as m and n increase, the requirement for time delay is decreased.

parameter	bifurcation value	period= $\frac{2\pi}{\omega}$
a	.27	20.2995
b	.8	26.5974
c	.02	20.2463
d	.08	24.6581

FIGURE 17. Periods of Oscillation at Hopf Bifurcations: The last column gives the period of oscillation in the system at the bifurcation values given in the second column. For each row in the table, all other parameters were held at the values in Figure 14.

of oscillation at this bifurcation. In each case, these calculations were made with all parameters taking on values from Figure 14 while setting the parameter in question at the specified bifurcation value. The values agree exactly with the numerical results of Scheper et al.

Conclusions

Mathematical models of biochemical systems which exhibit oscillatory behavior have often been based on Goodwin's negative feedback model. As a consequence of Bendixson's Criterion, two-variable systems oscillate only when there is both the autocatalytic production of one of the chemical components of the system and this negative feedback. However, in three variables, oscillation is possible based solely on the negative feedback of Goodwin's model. The addition of nonlinearity to this model creates a more chemically realistic situation by easing the unreasonably high level of cooperativity necessary for oscillation.

Although negative feedback has played the primary role in the explanation of circadian rhythms, there is evidence that positive feedback may be at work as well [6].

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